

CLAIMS

1. An isolated polypeptide of the SARS virus.
2. The polypeptide of claim 1, wherein the polypeptide is a Spike (S) polypeptide, an Env (E) polypeptide, a Membrane (M) polypeptide, a hemagglutinin-esterase polypeptide (HE), a
5 nucleocapsid (N) polypeptide, a ORF1a polypeptide, a ORF1ab polypeptide, a proteolytic fragment of a ORF1a polypeptide, or a proteolytic fragment of a ORF1ab polypeptide.
3. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO^S: 6039, 7232, 9766, 9767, 9768, 9769, 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 6042, 6043, 6044, 6045, 6046, 6047,
10 6048, 6049, 6050 or 6052.
4. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence having >75% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO^S: 6042, 6043, 6044, 6045, 6046, 6047, 6048, 6049, 6050, 6052, 9766, 9767, 9768, 9769, 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 9997, 9998, 10149, 10316,
15 10338, 10339, 10340, 10341, 10342, 10532, 10533, 10571, 10572, 10573, 10574, 10575, 10576, 10577, 10578, 10579, 11561, 11562, 11618, 11619, 11620, 11627, 11630, 11633 & 11636.
5. The polypeptide of claim 1, wherein the polypeptide comprises a fragment of at least 10 consecutive amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO^S: 6042, 6043, 6044, 6045, 6046, 6047, 6048, 6049, 6050, 6052, 9766, 9767, 9768, 9769,
20 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 9997, 9998, 10149, 10316, 10338, 10339, 10340, 10341, 10342, 10532, 10533, 10571, 10572, 10573, 10574, 10575, 10576, 10577, 10578, 10579, 11552, 11561, 11562, 11618, 11619, 11620, 11627, 11630, 11633 & 11636.
6. A polypeptide comprising an amino acid sequence having >80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO^S: 6042, 6043, 6044,
25 6045, 6046, 6047, 6048, 6049, 6050, 6052, 9766, 9767, 9768, 9769, 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 9997, 9998, 10149, 10316, 10338, 10339, 10340, 10341, 10342, 10532, 10533, 10571, 10572, 10573, 10574, 10575, 10576, 10577, 10578, 10579, 11552, 11561, 11562, 11618, 11619, 11620, 11627, 11630, 11633 & 11636.
7. A polypeptide comprising an amino acid sequence that comprises a fragment of at least
30 10 consecutive amino acids of an amino acid sequence selected from the group consisting SEQ ID NO^S: 6042, 6043, 6044, 6045, 6046, 6047, 6048, 6049, 6050, 6052, 9766, 9767, 9768, 9769, 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 9997, 9998, 10149, 10316, 10338, 10339, 10340, 10341, 10342, 10532, 10533, 10571, 10572, 10573, 10574, 10575, 10576, 10577, 10578, 10579, 11552, 11561, 11562, 11618, 11619, 11620, 11627, 11630, 11633 & 11636.

8. A polypeptide comprising an amino acid sequence having >80% sequence identity to SEQ ID NO: 6042, and/or comprising an amino acid sequence that comprises a fragment of at least 10 consecutive amino acids of SEQ ID NO: 6042, wherein the polypeptide is in the form of a trimer.
- 5 9. Nucleic acid encoding the polypeptide of any one of claims 1 to 8.
10. Nucleic acid according to claim 9, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO^S: 7191, 7273, 7275, 7277, 7279, 7281, 7283, 7285, 7287, 7289, 7291, 7292, 7293, 9968, 10066, 10084, 10299, 10505, 11323, 11563, 11639 & 11640.
- 10 11. A polynucleotide comprising a nucleotide sequence having >80% sequence identity to the nucleic acid of claim 9 or claim 10.
12. A polynucleotide comprising a fragment of at least 10 consecutive nucleotides of the nucleic acid of claim 9 or claim 10.
13. Antibody that recognizes the polypeptide of any one of claim 1 to 8.
14. The antibody of claim 13, wherein said antibody recognizes the polypeptide comprising
15 the amino acid sequence of SEQ ID NO: 6042 or a fragment thereof.
15. The antibody of claim 14, wherein said antibody recognizes the polypeptide comprising the amino acid sequence of SEQ ID NO: 6042 or a fragment thereof in trimeric form.
16. The antibody of claim 13, wherein the antibody is a monoclonal antibody,
17. The antibody of claim 13, wherein the antibody is a human antibody,
- 20 18. An immunoassay for detecting a SARS virus antigen in a sample, comprising the step of contacting the sample with the antibody of any one of claims 13 to 17.
19. An immunoassay for detecting an antibody against a SARS virus antigen in a sample, comprising the step of contacting the sample with the polypeptide of any one of claims 1 to 8.
20. A method of detecting an antibody against a SARS virus antigen in a sample comprising
25 contacting said sample with the polypeptide of any one of claims 1 to 8, under conditions suitable for binding said polypeptide to said antibody, if present, and detecting the binding of said polypeptide to said antibody.
21. A method for detecting a SARS virus antigen in a sample comprising contacting said
30 sample with the antibody of any one of claims 13 to 17, under conditions suitable for binding said antibody to said antigen, if present, and detecting the binding of said antibody to said antigen.

22. A vaccine for the treatment or prevention of severe acute respiratory syndrome (SARS), comprising an inactivated SARS virus, a killed SARS virus, an attenuated SARS virus, a split SARS virus preparation, or at least one purified SARS virus antigens.
23. The vaccine of claim 22, comprising a purified polypeptide according to any one of
5 claims 1 to 8.
24. The vaccine of claim 22 or claim 23, wherein the antigen is a purified SARS virus antigen in the form of a VLP.
25. The vaccine of any one of claims 22 to 24, further comprising an adjuvant.
26. The vaccine of claim 25, wherein the adjuvant is an aluminium salt or is MF59.
- 10 27. The vaccine of any one of claims 22 to 26, comprising more than one SARS virus antigen.
28. The vaccine of claim 27, wherein the antigens are selected from S, E, N and M.
29. The vaccine of claim 22, comprising an inactivated SARS virus.
30. The vaccine of claim 29, wherein said virus is inactivated by chemical or physical means.
- 15 31. The vaccine of claim 30, wherein said inactivation comprises treatment of the virus with an effective amount of one or more of the following agents selected from the group consisting of detergents, formaldehyde, formalin, β -propiolactone, and UV light.
32. The vaccine of claim 30, wherein said inactivation comprises treatment of the virus with an effective amount of one or more of the following agents selected from the group consisting of
20 methylene blue, psoralen and carboxyfullerene (C60).
33. The vaccine of claim 30, wherein said inactivation comprises treatment of the virus with an effective amount of one or more of the following agents selected from the group consisting of binary ethylamine, acetyl ethyleneimine and gamma irradiation.
34. The vaccine of claim 31, wherein said inactivation comprises treatment with β -
25 propiolactone.
35. The vaccine of claim 34, wherein said β -propiolactone is used at a concentration of 0.01 to 0.5%.
36. The vaccine of claim 34, wherein said β -propiolactone is used at a concentration of 0.5 to 0.2%.
- 30 37. The vaccine of claim 34, wherein said β -propiolactone is used at a concentration of 0.025 to 0.1%.

38. A method of inactivating SARS virus comprising exposing the virus to an inactivation agent for 12 to 24 hours at refrigeration temperatures followed hydrolysis of any residual inactivating agent by elevating the temperature for three hours.
39. The method of claim 38, wherein the inactivation agent is β -propiolactone.
- 5 40. The method of claim 38, wherein the refrigeration temperature is between 0°C and 8°C.
41. The method of claim 38, wherein the elevated temperature is between 33°C and 41°C.
42. A method for making an inactivated SARS vaccine comprising:
- a. innoculating a mammalian cell culture with SARS virus;
 - b. cultivating the infected cells;
 - 10 c. harvesting SARS virus containing supernatant;
 - d. inactivating the SARS virus; and
 - e. purifying the inactivated SARS virus.
43. The method of claim 42, wherein said mammalian cell culture is derived from one or more of the cell types selected from the group consisting of fibroblast cells, endothelial cells,
- 15 hepatocytes, keratinocytes, immune cells, mammary cells, smooth muscle cells, melanocyte cells, neural cells, prostate cells, renal cells, skeletal cells, liver cells, retinoblast cells and stromal cells.
44. The method of claim 42, wherein said mammalian cell culture is derived from a cell culture selected from the group consisting of human cells, non-human primate cells, HeLa cells,
- 20 human diploid cells, fetal rhesus lung cells, human embryonic kidney cells, VERO cells, horse cells, cow cells, sheep cells, dog cells, cat cells or rodent cells.
45. The method of claim 42, wherein said mammalian cell culture is derived from VERO cells or fetal rhesus kidney cells.
46. The method of claim 42, wherein said mammalian cells are cultured in serum free media.
- 25 47. The method of claim 42, wherein said mammalian cells are cultured in protein free media.
48. The method of claim 42, wherein said inoculating step comprising absorbing the SARS virus onto the cell culture for 60 to 300 minutes.
49. The method of claim 42, wherein said inoculating step is conducted at 25°C to 40°C.
- 30 50. The method of claim 42, wherein said purification step comprises one or more of the treatments selected from the group consisting of gradient centrifugation, ultracentrifugation, continuous-flow ultracentrifugation, chromatography, polyethylene glycol precipitation, and ammonium sulfate precipitation.

51. The method of claim 42, wherein said purification step comprises one or more of the treatments selected from the group consisting of ultrafiltration and dialfiltration.

52. The method of claim 50, wherein said chromatography treatment includes one or more of the chromatography treatments selected from the group consisting of ion exchange chromatography, size exclusion chromatography, and liquid affinity chromatography.

53. The method of claim 52, wherein said chromatography treatment includes use of one more chromatographic resins selected from the group consisting of an anionic resin and a cationic resin.

54. The method of claim 52, wherein the ion exchange chromatography treatment includes a first step using a strong anion exchange resin and a second step using a strong cation exchange resin.

55. The method of claim 50, wherein said gradient centrifugation purification step comprises density gradient centrifugation.

56. The method of claim 42, wherein said purification step comprises a first step of chromatography purification and a second step of gradient centrifugation.

57. The method of claim 56, wherein said first chromatography purification step comprises liquid affinity chromatography.

58. The method of claim 56, wherein said second gradient centrifugation step comprises density gradient centrifugation.

59. A single-stranded oligonucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 21-6020, 6076-6568, 6586-6587, 7292-7301, 7325-7328, 7332-7352, 7353-7385, 10235-10298, 10352-10504, 10580-11322 and 11325-11551.

60. A single-stranded oligonucleotide comprising the complement of the oligonucleotide of claim 59.

61. The oligonucleotide of claim 59 or claim 60, comprising 10-30 nucleotides.

62. The oligonucleotide of claim 61, comprising the nucleotide sequence of SEQ ID NO: 7292, SEQ ID NO: 7293, the complement of SEQ ID NO: 7292 or the complement of SEQ ID NO: 7293.

63. A kit comprising primers for amplifying a template sequence contained within a SARS virus nucleic acid target, the kit comprising a first primer and a second primer, wherein the first primer comprises a sequence substantially complementary to a portion of said template sequence and the second primer comprises a sequence substantially complementary to a portion of the

complement of said template sequence, wherein the sequences within said primers which have substantial complementarity define the termini of the template sequence to be amplified.

64. The kit of claim 63, wherein the template sequence is contained within SEQ ID NO: 1 and/or SEQ ID NO: 2.

65. The kit of claim 63 or claim 64, wherein the first primer comprises a fragment of 8 or more nucleotides of SEQ ID NO: 1, and the second primer comprises a fragment of 8 or more nucleotides of the complement of SEQ ID NO: 1.

66. The kit of claim 63 or claim 64, wherein the first primer comprises a fragment of 8 or more nucleotides of SEQ ID NO: 2, and the second primer comprises a fragment of 8 or more nucleotides of the complement of SEQ ID NO: 2.

67. The kit of claim 63, wherein the first primer is an oligonucleotide according to any one of claims 59 to 62 and the second primer is an oligonucleotide according to any of claims 59 to 62.

68. The kit of any one of claims 63 to 67, further comprising a labeled probe that comprises either a fragment of 8 or more nucleotides of SEQ ID NO: 1 and/or SEQ ID NO: 2, or the complement of said fragment, which fragment is located within the template sequence.

69. The kit of any one of claims 63 to 68, wherein the first primer and/or the second primer comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 21-6020, 6076-6568, 6586-6587, 7292-7301, 7325-7328, 7332-7352, 7353-7385, 10235-10298, 10352-10504, 10580-11322 and 11325-11551.

70. The kit of any one of claims 63 to 68, wherein the first primer and/or the second primer comprises the complement of a nucleotide sequence selected from the group consisting of SEQ ID NOS: 21-6020, 6076-6568, 6586-6587, 7292-7301, 7325-7328, 7332-7352, 7353-7385, 10235-10298, 10352-10504, 10580-11322 and 11325-11551.

71. A method of detecting the presence of SARS virus in a sample comprising providing a sample suspected of containing a SARS virus nucleic acid target, amplifying a template sequence contained within said SARS virus nucleic acid target with the kit of any one of claims 63 to 70, and detecting the amplified template sequence, wherein the presence of the amplified template sequence indicates the presence of SARS virus in said sample.

72. The method of claim 71, wherein said amplifying is accomplished using polymerase chain reaction, transcription mediated amplification, reverse transcription PCR, ligase chain reaction, strand displacement amplification or nucleic acid sequence-based amplification.

73. A double-stranded RNA molecule with a length from about 10 to about 30 nucleotides which is able to inactivate the SARS coronavirus in a mammalian cell.

74. The double-stranded RNA of claim 73, wherein the sequence of one of the strands is at least 90% identical to a target sequence, wherein the target sequence is a fragment of SEQ ID NO: 1 and/or SEQ ID NO: 2.

75. The double-stranded RNA of claim 73 or claim 74, wherein the target sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 7292, 7293, 7294, 7295, 7296, 7297, 7298, 7299, 7300 and 7301.

76. The double-stranded RNA of any one of claims 73 to 75, comprising at least one modified nucleotide.

77. A method for treating a patient suffering from SARS, comprising: administering to the patient a therapeutically effective dose of a molecule of less than 1000 g/mol.

78. The method of claim 77, wherein the molecule has an aromatic region and greater than one heteroatom selected from O, S, or N.

79. A method for treating a patient suffering from SARS, comprising: administering to the patient a therapeutically effective dose of a compound selected from: a nucleoside analog, a peptoid, an oligopeptide, a polypeptide a protease inhibitor, a 3C-like protease inhibitor, a papain-like protease inhibitor, or an inhibitor of an RNA dependent RNA polymerase.

80. A method for treating a patient suffering from SARS, comprising: administering to the patient a steroidal anti-inflammatory drug in combination with at least one antiviral compound.

81. A method for treating a patient suffering from SARS, comprising: administering to the patient a therapeutically effective dose of a compound selected from: acyclovir, gancyclovir, vidarabidine, foscarnet, cidofovir, amantidine, ribavirin, trifluorothymidine, zidovudine, didanosine, zalcitabine, an antiviral compound listed in Table 1; an antiviral compound listed in Table 2; or an interferon.

82. The method of claim 81, wherein the interferon is an interferon- α or an interferon- β .

83. The method of any one of claims 77 to 82, wherein the molecule or compound is delivered by inhalation.

84. A method of identifying a therapeutically active agent comprising the steps of: (a) contacting a therapeutically active agent with a cell infected with the SARS virus; (b) measuring attenuation of a SARS related enzyme.

85. A viral vector or particle for *in vivo* delivery of a nucleic acid of claim 9 or claim 10.

86. The viral vector of claim 85, wherein the vector is an adenovirus vector, a poxvirus vector or an alphavirus vector.

87. An alphavirus replicon particle comprising one or more SARS viral antigens.

88. The replicon particle of claim 87, wherein said SARS viral antigen is a spike protein.
89. The replicon particle of claim 87, wherein said particle comprises a replicon derived from Venezuelan Equine Encephalitis (VEE) and further comprises an envelope derived from Sindbus virus (SIN) or Semliki Forest Virus (SFV).
- 5 90. A vaccine comprising one or more SARS virus antigens and one or more respiratory virus antigens.
91. The vaccine of claim 90, wherein said respiratory virus antigens are selected from the group consisting of influenza virus, human rhinovirus (HRV), parainfluenza virus (PIV), respiratory syncytial virus (RSV), adenovirus, metapneumovirus, and rhinovirus.
- 10 92. The vaccine of claim 91, wherein said respiratory virus antigen is from influenza virus.
93. The vaccine of claim 90, wherein said respiratory virus antigen is from a coronavirus other than the SARS virus.
94. A polypeptide comprising an immunogenic, surface exposed fragment of the amino acid sequence SEQ ID NO: 6042.
- 15 95. The polypeptide of claim 94, wherein said fragment does not include the last 50 amino acids of the C-terminus of SEQ ID NO: 6042.
96. The polypeptide of claim 94, wherein said fragment does not include a transdomain region of SEQ ID NO: 6042.
97. The polypeptide of claim 94, wherein said fragment does not include a C-terminus
20 cytoplasmic domain of SEQ ID NO: 6042.
98. The polypeptide of claim 94, wherein said fragment does not include a N-terminus signal sequence.
99. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 9968 and 10066.
- 25 100. The polynucleotide of claim 99, wherein the polynucleotide comprising a nucleic acid sequence having > 80% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NOS: 9968 and 10066.
101. An isolated polynucleotide comprising a fragment of at least 15 consecutive nucleic acids of a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 9968 and 10066
30 and wherein said fragment does not consist entirely of SEQ ID NO: 10033.
102. An isolated polypeptide comprising an amino acid sequence encoded by any one of claims 99 – 101.

103. The polypeptide of claim 102, comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 9969 – 10032, 10067, and 10015.
104. The polypeptide of claim 103, wherein the amino acid sequence is selected from the group consisting of SEQ ID NOS: 9997, 9998 and 10015.
- 5 105. An expression construct for recombinant expression of a SARS virus spike protein wherein said construct comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 6578 – 6583.
106. A mammalian cell line stably expressing a SARS viral antigen.
107. The cell line of claim 106, wherein said cell line is a Chinese Hamster Ovary (CHO) cell.
- 10 108. The cell line of claim 106, wherein the SARS viral antigen is a spike protein or fragment thereof.
109. The cell line of claim 106, wherein the spike protein is truncated to remove the transmembrane sequence.
110. A method of identifying a therapeutically active agent comprising the steps of: (a)
15 contacting a therapeutically active agent with a buffer comprising SARS enzyme; and (b) measuring attenuation of the SARS enzyme.
111. The method of claim 110 wherein the SARS enzyme is a SARS protease.
112. The method of claim 111 wherein the buffer further comprises a peptide with a SARS protease cleave site.
- 20 113. The method of claim 110 wherein the measurement is made by the measurement of fluorescence.
114. A vaccine of one of claims 22 to 37, and 90 to 93 further comprising an adjuvant.
115. The vaccine of claim 114 wherein the adjuvant is a SMIP.
116. The vaccine of claim 115 wherein the SMIP compound is selected from the group
25 consisting of an acylpiperazine, a tryptanthrin, an indoleione, a tetrahydroisoquinoline, a benzocyclodione, an amino azavinyl compound, a thiosemicarbazone, a lactam, an aminobenzimidazole quinolinone, a hydrophthalamide, a benzophenone, an isoxazole, a sterol, a quinazolinone, a pyrole, an anthraquinone, a quinoxaline, a triazine, an benzazole, and a pyrazolopyrimidine, or a pharmaceutically acceptable salt, ester, or prodrug thereof.
- 30 117. A method of vaccinating a subject comprising administering a vaccine of one of claims 22 to 37, and 90 to 93.
118. The method of claim 117 further comprising administering a SMIP.

119. A method for treating a patient of one of claims 77 to 82 further comprising administering at least one SMIP compound.

120. A method for treating a patient of one of claims 77 to 82 further comprising administering at least one SMIS compound.